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ScienceDirect

Procedia Engineering 120 (2015) 92 – 95

Procedia Engineering

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EUROSENSORS 2015

Air-droplets as Gas Reservoir to Provide O_2 to the Stored-Aqueous Droplets in Micro-channels

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Abstract

We present a method to supply oxygen to on-chip stored aqueous droplets surrounded by oxygen-permeable fluorinated oil in micro-channels. By generating air-droplets (air-bubbles) in between the aqueous droplets, oxygen diffuses to those aqueous droplets. This is confirmed by experiments comparing the effect of nitrogen bubbles and air-bubbles. The proposed method and device are a step towards long-term on-chip cultivation of mammalian cells and aerobic bacteria growth in droplets.

Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

Keywords: droplet storage, oxygen feeding, fluorinated oil, micro-fluidics

1. Introduction

The analysis of cells in droplets is gaining importance because of the extremely small sample volume, the possibility of isolation and compartmentalization of single cells, and the ability of high-throughput analysis. The droplet is the equivalent of the test tube, with droplet volumes in the femto-to nanolitre range, also yielding significant reduction in instrumental footprints. To culture mammalian cells or to promote the growth of aerobic bacteria, droplets need to be provided with sufficient oxygen.

One way to supply oxygen to droplets is to use porous substrate materials like polydimethylsiloxane (PDMS) through which the gas can diffuse to the microfluidic channel. However, the liquids in the channels also diffuse

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Peer-review under responsibility of the organizing committee of EUROSENSORS 2015
doi:10.1016/j.proeng.2015.08.573

through the porous substrate decreasing the volume of the droplets severely. It was shown that PDMS channels coated with 2µm parylene-AF4 suppresses the evaporation of aqueous droplets in micro-channels drastically [1] but it also blocks the diffusion of oxygen through the PDMS into the channels, making long-term cultivation of mammalian cells impossible.

An existing technique to provide oxygen in microfluidic channels is based on thin oxygen permeable PDMS layers separating aqueous channels from oxygen feeding gas channels [2]. Devices using this technique consist of multiple layers making them hard to realize because of the fragility of the thin PDMS membrane and the need for multiple lithography steps. Another technique to supply oxygen to droplets is based on an off-chip (in a reservoir) droplet storage method [3,4]. This method does not allow continuous monitoring of cell growth because for the analysis the droplets have to be transferred back into the microfluidic chip.

Here we present a single-layer droplet-based microfluidics platform that allows to culture cells in droplets. The cells are supplied with oxygen from stored air-bubbles suspended in fluorinated oil. To our knowledge, this is the first time this bubble-to-droplet oxygen transfer is used.

2. Materials and Methods

2.1. Chip Design and Fabrication

The microfluidic platform should allow the creation of droplets in which the cells can be transported and cultured, and the creation of oxygen containing gas-bubbles to feed the droplets with oxygen over time. In Fig. 1(a) a schematic of the microchannel layout used in our experiments is shown. A flow of gas-bubbles suspended in fluorinated oil is created at the W-junction of the top three inlets. Air-bubble contact with the channel wall, which might hinder the flow, is prevented by controlling the flow-rates of the oil and gas inlets. In the following T-junction the liquid droplets are generated in between the gas-bubbles. By adjusting the flow rates for the W-junction and the T-junction, the formation of the aqueous droplets between gas-bubbles is easily achievable.

The microchannel (width: 100µm, height: 100µm and length: 12mm) was realized by bonding a structured PDMS layer on a glass slide (standard soft lithography). The photograph of fabricated microfluidic device is depicted in Fig. 1(b).

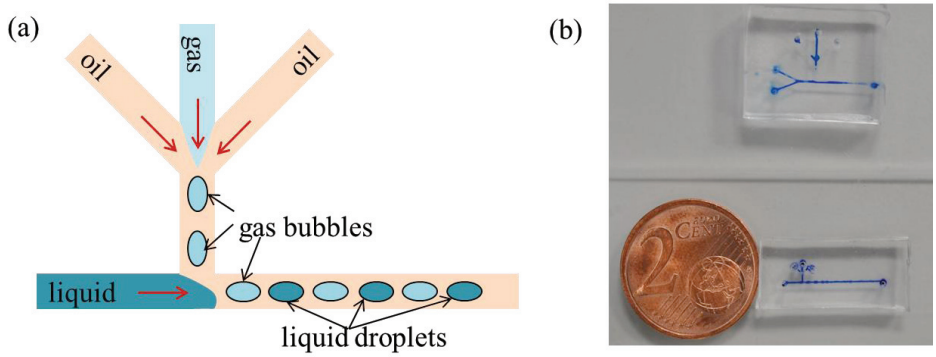

Fig. 1. Schematic of the chip layout to generate gas-droplets in between aqueous droplets in continuous oil flow. Flow-focusing generates gas-droplets in oil and brings them to the straight channel in aqueous droplets (a). Photograph of the fabricated PDMS channel sealed on a glass slide. The channel was filled with blue dye for better visualization (b).

2.2. Fluorinated Oil

FC-40 (Sigma-Aldrich, Germany) fluorinated oil is used as carrier liquid and also as an oxygen permeable interface between air-bubbles and aqueous droplets. Fluorinated oil has a 20 times higher oxygen solubility and a

three times higher CO_2 solubility than pure water making the culture of living cells encapsulated in aqueous droplets particularly easy.

2.3. Methylene Blue as Oxygen Indicator

Methylene blue (methyl thionine chloride) is a dye which reversibly changes its color upon oxidation and reduction. Methylene blue is reduced by a reductant (i.e. alkaline solution of glucose) to form a colorless leucomethylene blue. It can turn back to methylene blue by oxidation. To demonstrate the presence of oxygen in the micro-droplets, methylene blue solution was mixed in the channel with the solution of glucose and sodium hydroxide.

3. Results and Discussion

Fig. 2 shows the generation of air-bubbles and water droplets in oil. The air-bubbles suspended in oil were generated at the W-junction and carried to the T-junctions by a pressure driven flow. Blue dye was introduced at the left-hand side of the T-junction creating a mix of aqueous droplets and gas-bubbles suspended in fluorinated oil.

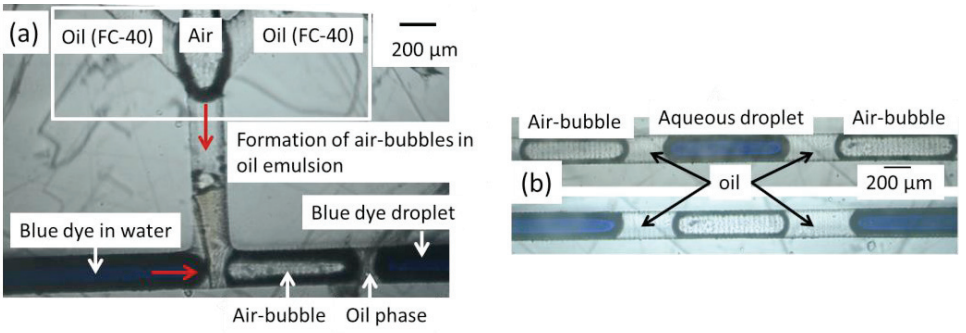

Fig. 2. Formation of air-bubbles in oil uses a W-junction and formation of blue dye droplets in oil (FC-40) uses a T-junction (a). Bluedye-oil-air droplets are stored in the straight micro-channel (b). Blue dye in water was used for better visualization.

The diffusion of oxygen in a micro-channel was demonstrated by mixing the redox indicator methylene blue and a solution of glucose and sodium-hydroxide at equal flow rates (each 30µl/hr) in the cross-section of a T-junction. Fig. 3 shows that the methylene blue is becoming colorless with the travelling distance (time) as dissolved oxygen is consumed by the alkaline glucose solution.

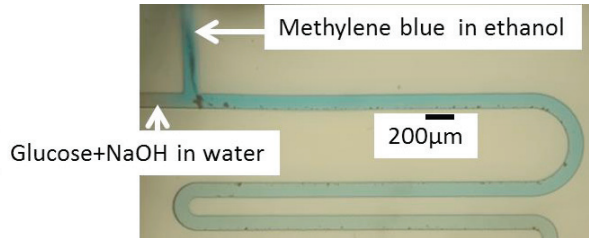

Fig. 3. The reaction of methylene blue with a solution of glucose (2.5%) and NaOH (2.5%) in DI water is shown in a micro-channel. The channel was flushed with oil to prevent entrapment of air bubbles. The solution took only 30 sec to become colorless due to the absence of oxygen in the channel.

In Fig. 4 it is shown how the presence of air-bubbles increases the level of oxygen in the aqueous droplet. On the left the bubbles contained pure nitrogen, on the right they contained air. The methylene blue in alkaline glucose

solution droplets takes 2 only minutes to become colorless in the presence of nitrogen bubbles (a); where as it needs 30 minutes in the presence of air-bubbles (b).

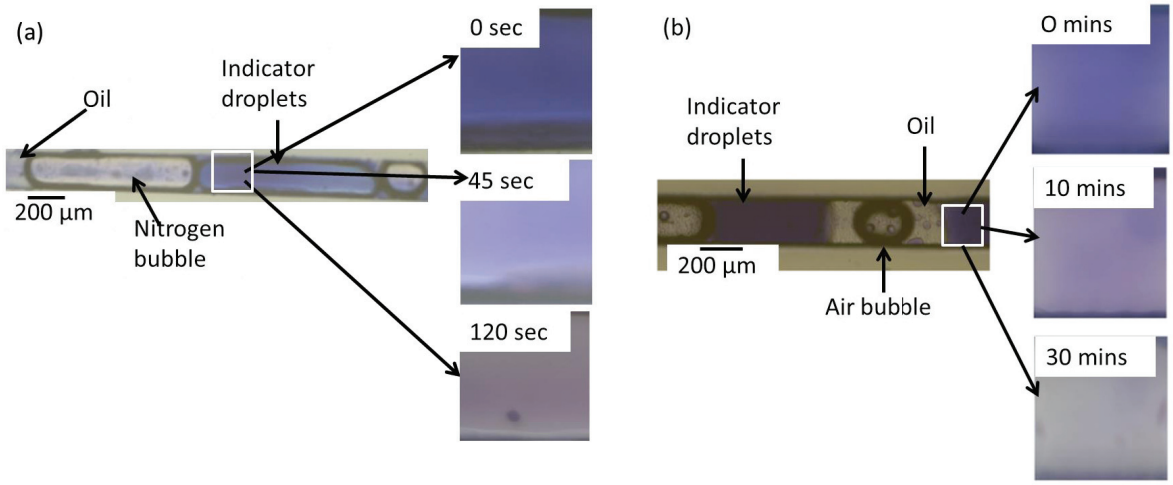

Fig. 4. Detection of oxygen in aqueous droplets in presence of nitrogen-bubbles and air-bubbles suspended in fluorinated oil. There is a significant color change in terms of time. Methylene blue in alkaline glucose solution droplets takes 2 only minutes to become colorless in the presence of nitrogen bubbles (a); where as it needs 30 minutes in the presence of air-bubbles (b).

4. Conclusions

We have designed and realized a multiphase microfluidic system that enables the feeding of oxygen from air-bubbles to aqueous droplets without the need of changing the medium. The diffusion of oxygen from air-bubbles to aqueous-droplets suspended in fluorinated oil was successfully demonstrated using methylene blue as indicator. The proposed method and device are a step towards long-term on-chip cultivation of mammalian cells and aerobic bacteria growth in stored droplets.

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